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MEDIATORS OF FEVER AND MUSCLE PROTEOLYSIS

THE accelerated catabolism of skeletal-muscle protein that accompanies severe trauma or infection can now be ascribed to the actions of endogenously produced mediators. Two noteworthy studies described[1,2] in this issue of the *Journal* have identified these actions by using in vitro preparations of rat skeletal muscle in a bioassay. Rates of proteolysis in muscle preparations were determined by measuring the release of free amino acid into the medium.

Clowes et al.[1] isolated and partially characterized a small glycopeptide from the plasma of patients with sepsis or trauma. When compared with control samples of normal plasma, samples containing the mediator were found to induce significantly higher rates of muscle proteolysis. Intermediate rates of amino acid release were generated by plasma from patients who had undergone uncomplicated elective surgery. In addition, the magnitude of in vivo proteolysis in the uninjured leg muscles of patients with sepsis or trauma was estimated by measuring arteriovenous differences in the plasma concentrations of representative free amino acids. Leg-muscle proteolysis measured in this way in individual patients correlated well with their plasma bioactivity in the assay system.

Although the evidence for a circulating proteolytic mediator was indirect in the study of Clowes et al.,[1] additional support for the presumptive role of circulating factors was found by Baracos et al.,[2] who studied the actions of highly purified human leukocytic pyrogen in a similar rat-muscle bioassay system. Leukocytic pyrogen produced a rapid increase in muscle proteolysis without affecting the synthesis of new muscle protein. Baracos et al. also showed that proteolysis induced by this pyrogen was mediated through the synthesis of prostaglandin E_2 in muscle. Both the accumulation of prostaglandin E_2 and the proteolytic action of leukocytic pyrogen could be blocked by incubation of muscle with indomethacin, a drug known to inhibit the synthesis of prostaglandin E_2. Baracos et al.[2] also demonstrated that the acceleration of muscle proteolysis induced by leukocytic pyrogen could be blocked through a different mechanism by an experimental drug, Ep-475, which specifically inactivates the lysosomal cathepsins B, H, and L in intact skeletal muscle. The findings suggested that the proteolytic actions of leukocytic pyrogen in skeletal muscle were caused by increased production of prostaglandin E_2, which in turn activated thiol proteases in muscle-cell lysosomes.[2] Alternatively, naturally occurring cathepsin inhibitors in muscle[3] may have been deactivated.

Endogenous peptide mediators are formed and released when mobile phagocytic cells are suitably stimulated. Fever induced by leukocytic pyrogen is mediated in hypothalamic thermal regulatory centers by local formation of prostaglandin E_2 in neuronal cells.[4] The action of leukocytic pyrogen on skeletal muscle would thus appear to employ the same secondary messenger. Further work will be required to determine whether the glycopeptide mediator identified in plasma by Clowes et al. is structurally related to leukocytic pyrogen, whether it is produced by activated phagocytes, and whether it works by a similar molecular mechanism.

These new findings[1,2] extend the concept that activated phagocytic cells can produce hormone-like mediators to signal distant tissues. Mediator activities are reflected by a variety of names,[5] including "leukocytic pyrogen," "endogenous pyrogen," "leukocytic endogenous mediator," "neutrophil-releasing factor," "lymphocyte-activating factor," and most recently "interleukin-1." However, the molecular structures of these mediators are not known, and their interrelations are uncertain.

Many of the generalized but diverse metabolic and physiologic responses that accompany severe trauma, infection, or inflammatory states have been ascribed to the actions of endogenous mediators.[5,6] Such responses include the experimentally demonstrated generation of fever, the production and release from bone marrow of neutrophils, the accelerated hepatic uptake of amino acids from plasma, the hepatic synthesis of intracellular enzymes and metal-binding proteins, the hepatic production of acute-phase plasma proteins, the hepatic sequestration of iron and zinc, and the stimulation of phagocyte and lymphocyte populations to increase their activity.[5,6] Mediator activities that can be demonstrated in cultured cells or tissue preparations appear to be independent of intervening neural or hormonal controls.

Acceleration of skeletal-muscle proteolysis was previously included in this list of mediator-induced responses on theoretical grounds.[5] It seemed logical to suppose that mediator release might activate a mechanism for generating the free amino acids needed for host defenses. It remains possible that another consistent response to illness — i.e., anorexia — is also initiated by an endogenous mediator.

Muscle proteolysis during severe illness is of value for survival. Skeletal muscle provides a metabolically dynamic protein bank and potential source of free amino acids.[7,8] This role of skeletal-muscle protein is beneficial because both immunologic and nonimmunologic host-defense mechanisms are ultimately based on the ability of body cells to synthesize new proteins. With severe trauma, infection, or inflammation, the labile source of amino acids in muscle can be tapped for the high-priority defensive needs of the host. On the other hand, if the pool of labile nitrogen becomes depleted, as in cachectic diseases or severe protein malnutrition, the patient becomes especially vulnerable to superimposed infections, often by opportunistic microorganisms.[7]

In addition to the amino acids reused for synthesis of new proteins, branched-chain amino acids released during proteolysis can be metabolized within muscle as direct sources of energy. Other amino acids, similarly released or synthesized within muscle cells, travel through plasma to the liver, where they may become substrates for gluconeogenesis.[7] The additional glucose is used in turn to initiate and sustain the heightened consumption of oxygen that accompanies fever. The two reports in this issue should stimulate comparisons of muscle proteolysis due to various cachectic illnesses, to glucocorticosteroids and other hormones,[8] and to previously identified muscle-protease regulators.[3] The responses of cardiac muscle must also be compared with the responses of striated skeletal muscle.

Unfortunately, mediator substances have not been

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available in sufficient purity or quantity to allow broad testing in all the bioassay systems in which some of their activities have been explored. The possibility that a single mediator initiates all recognized host responses seems remote in view of the physicochemical and immunologic differences between partially purified species of leukocytic pyrogen.[9] Furthermore, a single mediator could not account for the disease-related differences in clinical fever patterns, leukocyte responses, and acute-phase protein fluctuations. However, since laboratory production of various mediators employs similar cells and methods, it is possible that different species of mediators are all members of a closely related family.[6]

Despite more than three decades of study, these endogenous mediators have not been precisely identified or characterized. Formidable obstacles to progress remain. The need to obtain mediators in vitro from living phagocytic cells limits the size of production runs. Biological characterizations still depend on relatively insensitive bioassay systems. Large mediator losses occur during purification and standardization. Specific structural characterization and workable quantities of mediators will be required to determine individual physiologic roles.

Perhaps these obstacles can be overcome. Recently, Flynn et al.[10] identified mononuclear phagocytes in human placentas as sources for mediator production. Recombinant-DNA technologies offer a possible method for future production. Bioassay systems should be made more sensitive and should use cultured tissue or cells to minimize the loss of purified mediator, or they should be replaced by physicochemical quantitation or immunoassay. The latter technique should improve when well-characterized mediators and specific high-affinity polyclonal or monoclonal antibodies are produced.

A full understanding of the general response to injury and infection still eludes us, but the two reports in today's issue represent an important step forward. They give promise of an early elucidation of the molecular mechanisms underlying the breakdown of muscle protein, which is such a prominent feature of that response.

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